In vivo DNA nanostructure synthesis for disease screening

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Abstract

DNA has emerged as a promising material for the creation of novel functional nanostructures. Here we present DNA nanostructures capable of simultaneous detection of multiple microRNA (miRNA) targets which are identified as promising disease biomarkers. Logic gates can be easily incorporated into our designs to test various combinations of miRNA targets. G-quadruplexes form when the specified target hybridizes with the probe, generating fluorescence in the presence of substrate. We endeavor to demonstrate intracellular synthesis, self-assembly and functioning of our nanostructures inside *E. coli*. Our constructs open up new possibilities in future research on DNA nanotechnologies as diagnostic tools, and promote the applications of miRNA testing in clinical conditions.

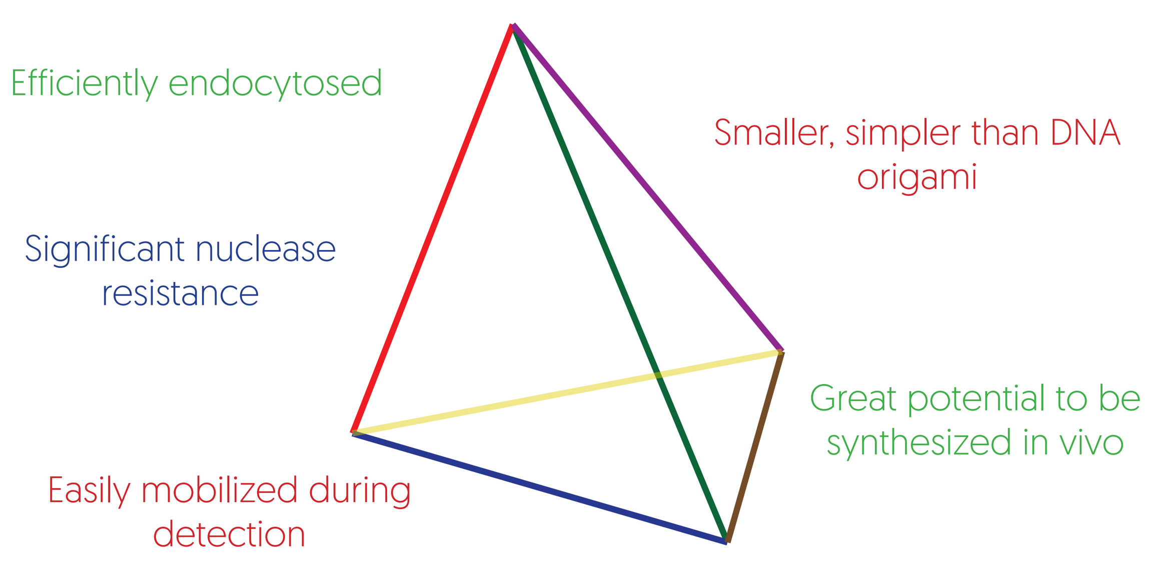
Design rationale

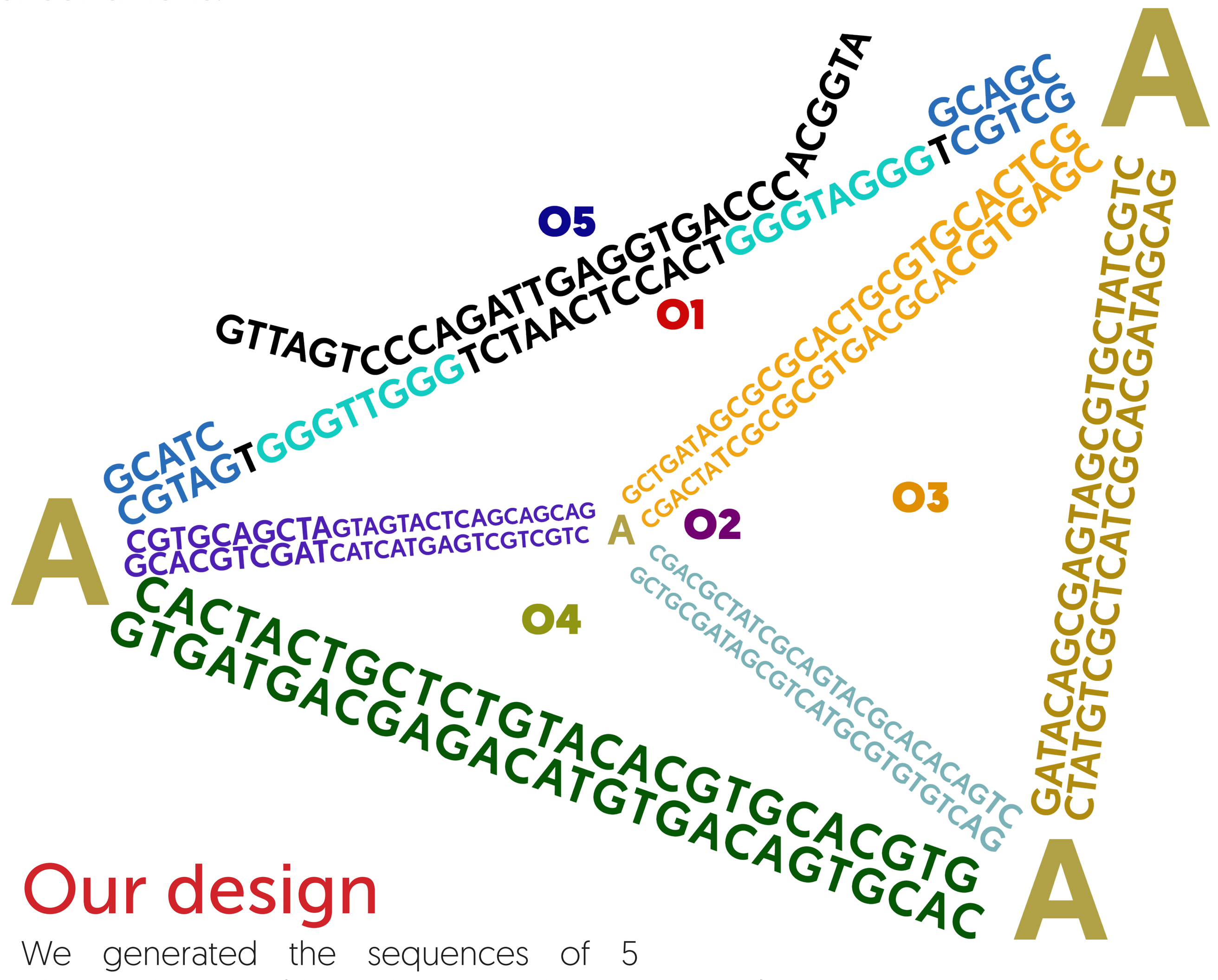
miRNA

microRNAs are important biomarkers in our body. For example, miR-34b have elevated serum levels before onset of symptoms for huntington's disease. miR-15b-5p, miR-338-5p and miR-764 are also significantly over-expressed in plasma of patients with hepatocellular cancer.

Tetrahedron

Tetrahedrons have various advantages over other nanostructures. The following lists some of them.





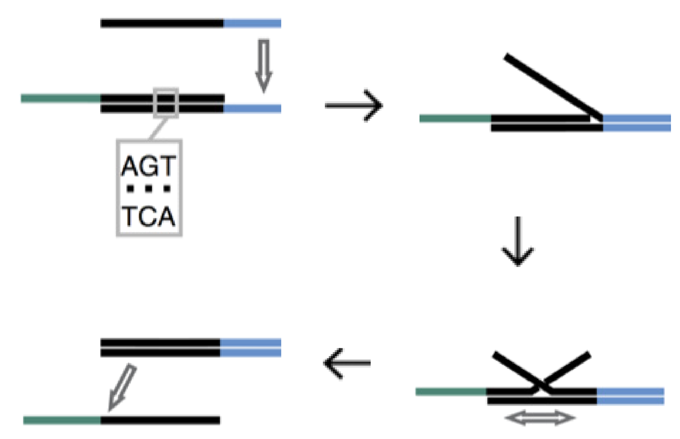
Our design

We generated the sequences of 5 oligonucleotides (O1 to O5 in the diagram above) that can be self-assembled into a tetrahedron using Tiamat, a software for DNA sequence generation. Base pairing between the oligonucleotides are shown in the above diagram.

System Mechanism

Strand displacement

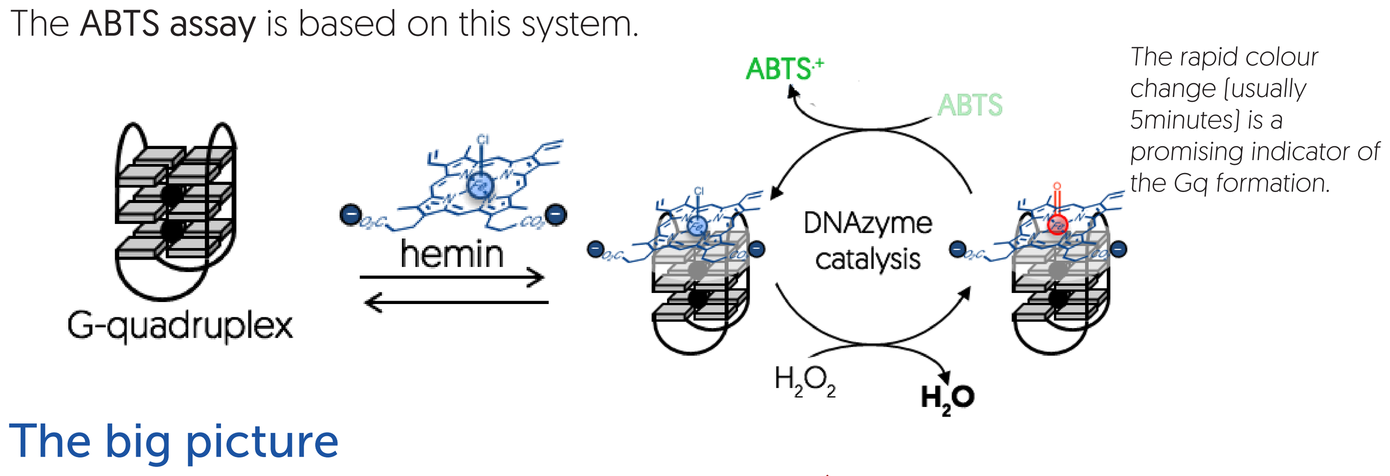
Two strands with partly or fully complementary sequences hybridize to each other at an unhybridized region called toehold, displacing one or more prehybridized strands.



Split G-quadruplex

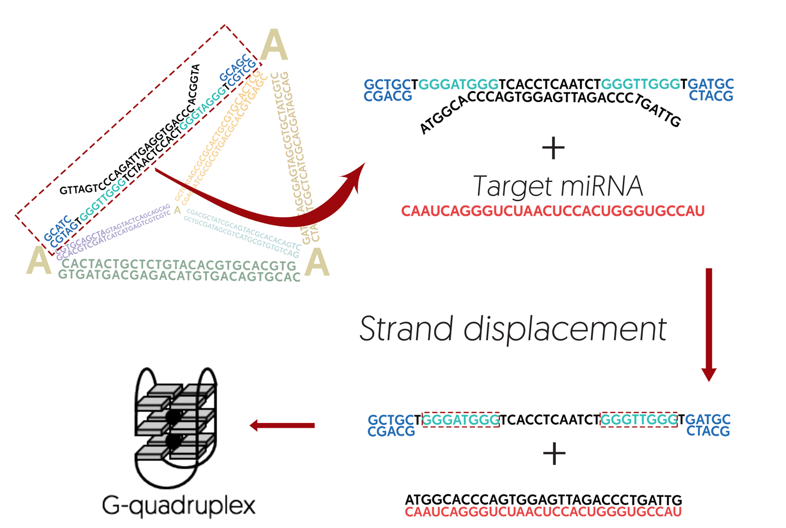
G-quadruplex (Gq) are tertiary structures formed by sequences rich in guanine. When Gq forms a complex with hemin, it exhibits peroxidase activity and functions like a DNAzyme. Its catalytic activity is utilized in many DNA nanostructures where a colour change is produced by target-induced conformational change.

The ABTS assay is based on this system.

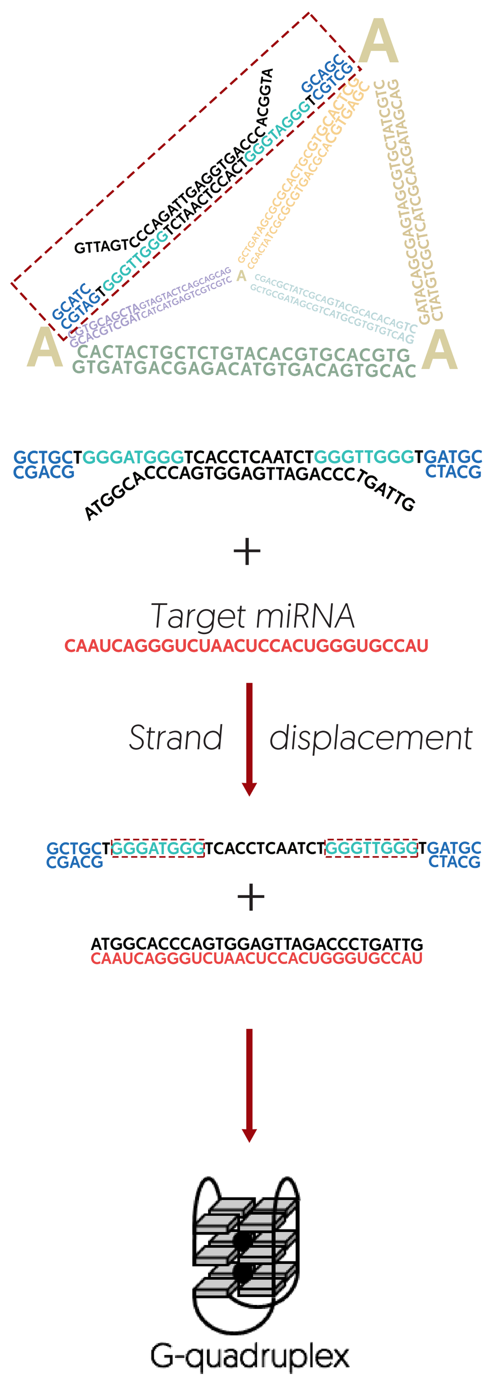


The big picture

Putting strand displacement, tetrahedron and Gq principles together yields the final product. A target miRNA binds to the toehold of the tetrahedron, displacing the prehybridized strand, forming Gq with peroxidase activity.

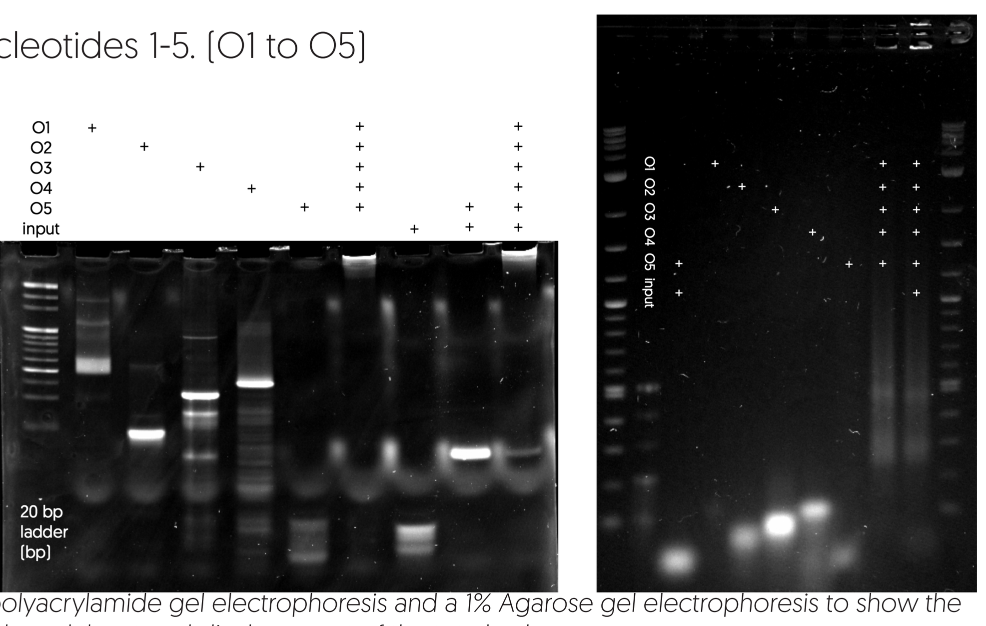


Results and discussion

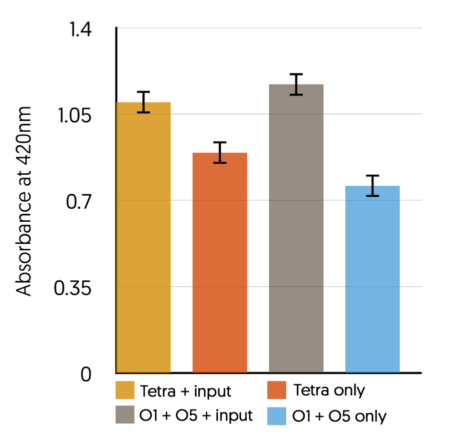


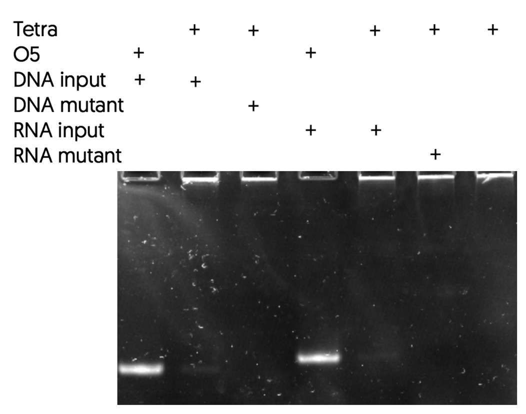
We assembled the tetrahedron by mixing equal molar of oligonucleotides 1-5. (O1 to O5)

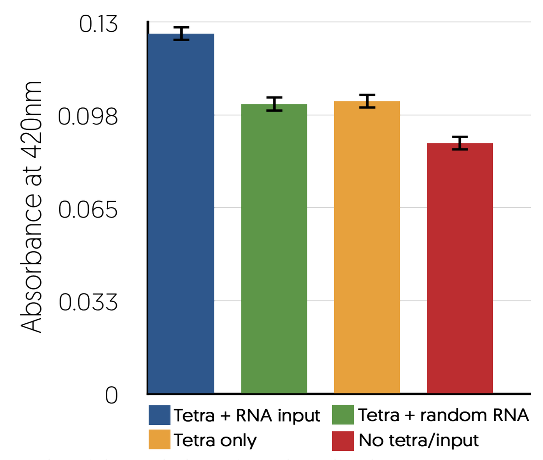
The DNA/RNA input strand, which is of 30nt and is based on the miRNA biomarker of Hungtington disease miR34b) was put in equal molar with the tetrahedron for strand displacement and the formation of Gq. The product from the displacement yields a double stranded O5+input dimer of 30bp. After considerable number of runs with DNA, RNA was used as the input, otherwise constant.



A 12% polyacrylamide gel electrophoresis and a 1% Agarose gel electrophoresis to show the assembly and the strand displacement of the tetrahedron.

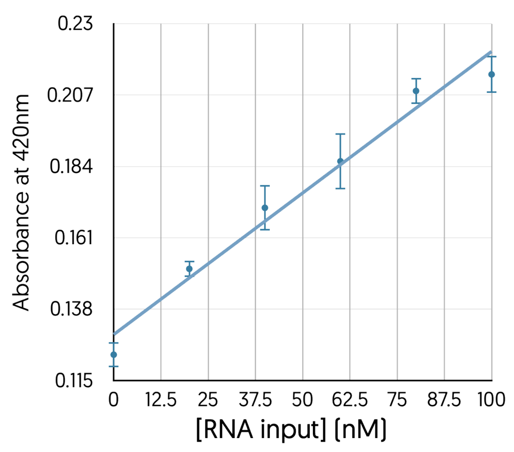






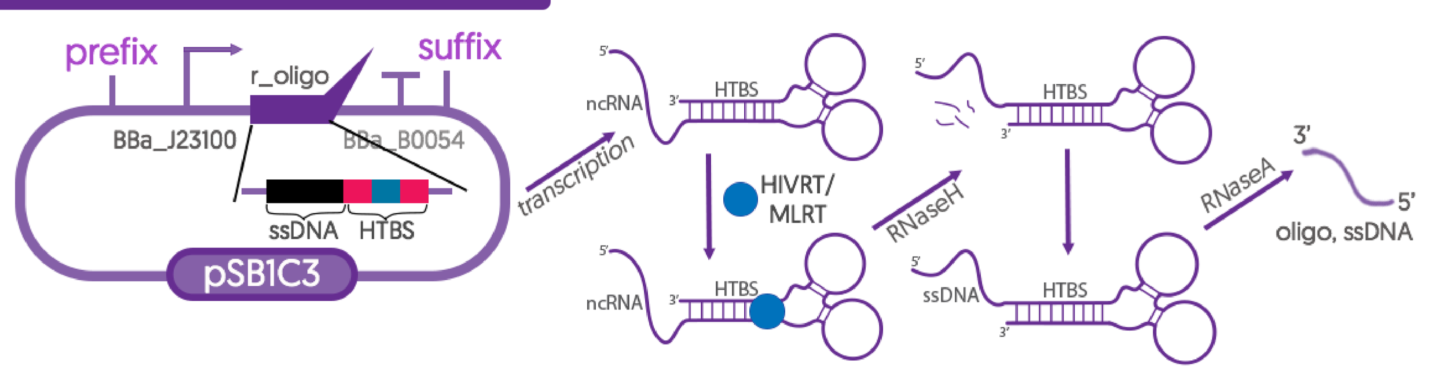
A bar chart showing the Gq formation of simplified beacon and the tetrahedron done under the same condition (left); a 12% polyacrylamide gel showing the displacement between DNA and RNA input, with output markers of DNA/RNA input + O5 (middle); A graph showing the differences between the absorbance measured at 420nm at 15-minutes with different component added (right).

To test the sensitivity of our probe, an limitation of detection analysis was conducted by varying the concentration of DNA & RNA input from 0nM to 100nM. LOD was calculated from the standard error of the regression line $y=0.0009x+0.1298$ ($R^2=0.9739$). LOD=17.2nM.



Plasmid construction

Our featured composite parts (BBa_K205400 and BBa_K2054006) encode for all the ssDNAs for our desired nanostructure and the active component respectively from one single device. Whereas our basic parts (BBa_K2054001 - BBa_K2054005) each contains a 'r_oligo' region that encodes together with reverse transcriptase, separate single-stranded DNA - our oligos 1 - 5.

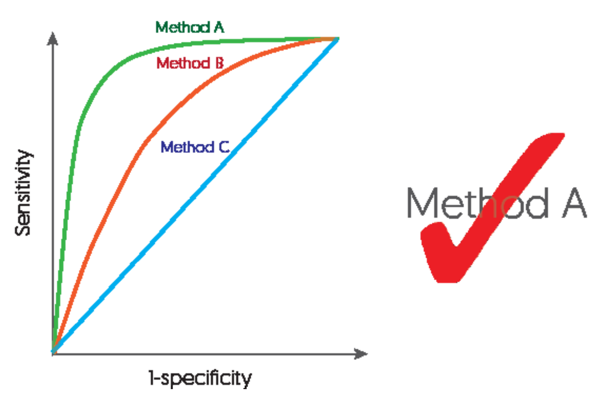


The 'r_oligo' region transcribes a product containing a non-coding RNA (ncRNA) and a HIV-Terminator-Binding Site (HTBS) that exhibit a 3'-hairpin structure. The HTBS serves as a terminator in this gene, where the HIV reverse transcriptase (RT) binds. During reverse transcription, the binding of HIVRT initiates the elongation, aided by another RT murine leukemia reverse transcriptase (MLRT). RNase H cleaves specifically the ncRNA-DNA linkages, leaving the desired ssDNA, attached to the HTBS on its 5' end, where RNase A breaks to release.

Future Work

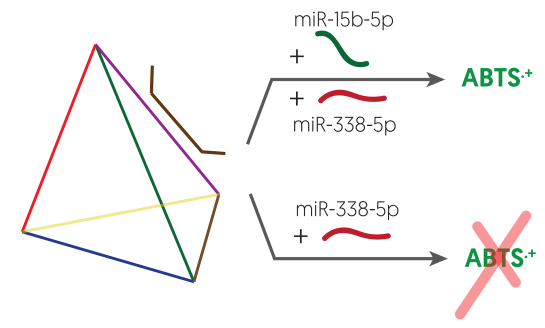
We will be using transmission electron microscopy to visualize the tetrahedron for a complete verification of formation of the correct tetrahedral shape.

Optimization is another aspect that is hindering the efficiency of the production of a specific and sensitive device. A software could be made based on previous findings to aid in optimization.

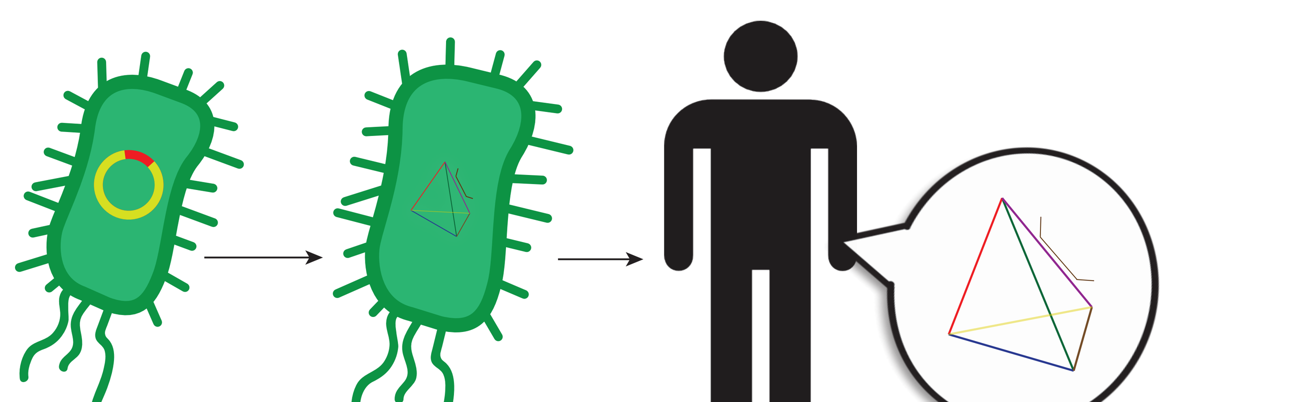


After LOD, receiver operating characteristic (ROC) curve could also be plotted to see if the best point matches the clinical definition of related diseases. This could also be compared with other ROC curves from other diagnostic methods.

We are also planning to incorporate the concept of logic gates into the design to increase specificity and to expand the design's scope of diagnosing.



Of course, the final step we hope to achieve is to get the functional 3D nanostructure synthesised in-vivo and apply to the diagnostic world.



WikiPage: 2016.iegm.org/Team:Hong_Kong_HKU Facebook: HKU iGEM 2016 Team Twitter: hku_igem_2016

Team members: Lai Hei Wai, Lau Yui Ching, Ho Sin Ning Shannon, Tam Ka Ki Tim, Lam Yung Shing, Yung Canaan, Law Janice Hiu Ching, Lo Yik Hei, Na Ri Sha, Dissanayake Thirmendra, Chiu Man Ying. Special thanks to LKS faculty of Medicine, The University of Hong Kong, Dr. Julian A. Tanner, Mr. Simon Shiu and Mr. Jacky Yuen for their continuous support, including constant advising, directions-giving and technical support. Some of the diagrams were edited from Stephen Loic, et.al. (2016).

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